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GENOTYPE DIVERSITY OF *SALSOLA TRAGUS* AND POTENTIAL ORIGINS OF A PREVIOUSLY UNIDENTIFIED INVASIVE *SALSOLA* FROM CALIFORNIA AND ARIZONA

JOHN F. GASKIN
USDA Agriculture Research Service, Northern Plains Agricultural Research Laboratory, 1500 N. Central Avenue, Sidney, MT 59270
jgaskin@sidney.ars.usda.gov

FREDERICK J. RYAN
USDA Agricultural Research Service, Crop Diseases, Pests, and Genetics Research Unit, 9611 South Riverbend Avenue, Parlier, CA 93704

G. FREDERIC HRUSA
California Department of Food & Agriculture, Botany Laboratory & Herbarium, Plant Pest Diagnostics Center, 3294 Meadowview Road, Sacramento, CA 95832

JASON P. LONDO
Department of Biology, Washington University in St. Louis, Campus Box 1137, One Brookings Drive, St. Louis, MO 63130

ABSTRACT

Various *Salsola* spp. have been introduced to the USA, and some of these are considered noxious or invasive in certain regions. The genus is taxonomically challenging, and recent morphological, cytological, and molecular work has shown that an unknown taxon, previously identified as *S. tragus*, but recently temporarily designated as Type B, with unknown origins, exists in California and Arizona. Type B's origins and identity are a point of concern for biological control efforts, as it is much less affected by fungal biological control agents than the sympatric invasive *S. tragus*. Initial studies in 2005 indicated that Type B is morphologically very similar to *S. kali* subsp. *austroafricana* which has been collected in southern Africa. Here we use DNA sequence data from a PEPC intron to compare USA and Old World specimens, and results indicate that *S. kali* subsp. *austroafricana* is genetically identical to Type B and distinct from *S. tragus*. It is unclear if *S. kali* subsp. *austroafricana* is native to southern Africa or to the northern hemisphere of the Old World, and further investigations in both regions are needed.

Key Words: PEPC, internal transcribed spacer, invasion, molecular systematics, Russian thistle, tumbleweed.

The native range of *Salsola tragus* L. (Russian thistle, family Chenopodiaceae) extends from northeastern China to the Atlantic coast of Europe and includes northern Africa (Rilke 1999). In North America, *S. tragus* was introduced into South Dakota in the 1870's, has spread throughout much of the USA and Canada, and is considered a noxious weed in California and Colorado.

Other *Salsola* species have been introduced to North America at various times (Mosyakin 1996; Mosyakin 2003; USDA 2005), including: *S. kali* L., *S. collina* Pall., and *S. paulsenii* Litv. of section *Kali* Dumort.; *S. soda* L. of section *Salsola* s.s.; and the more distantly related *S. vermiculata* L. of section *Caroxylon* (Thunb.) Fenzl.

Species level nomenclature within *Salsola* sect. *Salsola* s.l. has undergone many revisions and numerous alternate applications. Rilke (1999) has recently proposed a revision of the section; in North America this includes *S. collina*, *S. kali*, *S. paulsenii*, *S. tragus* and *S. soda*. Wilken (1993) and Mosyakin (1996) list several names that have been applied or misapplied to *S. tragus* in North America, while Rilke (1999) lists more than fifty synonyms for Old World *S. tragus* alone.

Hybridization between species within this section apparently has occurred in their native range. Intermediate forms, such as between *S. paulsenii* and *S. tragus*, extend over a wide geographic area in central Asia (Rilke 1999, p. 159). Rilke suggested that such zones may occur in North America as well (Rilke 1999, p. 164), although her monograph specifically states that few North American specimens were observed during its preparation. Other authors have also suggested the possibility of interspecific hybridization among *Salsola* species in North America (Arnold 1973; Beatley 1973; Wilken 1993). Experimental evidence, however, was not presented.

Against this background of taxonomic and genetic commotion, there are only a limited number of reliable morphological characters available to distinguish the closely related species of sect. *Salsola* s.l. For example, the most recent *Salsola* treatment for California (Wilken 1993) primarily used tepal-wing presence and diameter, plus stem pubescence, to separate three species in two sections s.s., while Rilke (1999) also used individual tepal-wing dimensions and anther length to separate ten species of subsection *Kali*.

DNA sequence data has also been used to examine phylogenetic relationships in *Salsola*. Pyankov et al. (2002) used the Internal Transcribed Spacer (ITS) of 18S-26S ribosomal DNA, as well as anatomical and physiological characters, to examine 34 *Salsola* and allied species from Europe and Asia. Most-parsimonious analysis of the ITS data grouped *S. kali* and *S. paulsenii* together in a single clade within a multiclade group possessing NADP-malic enzyme C4 photosynthesis, in agreement with analyses of *Salsola* based on anatomical characters (Botschantsev 1969; Freitag 1997). Samples from North America were not examined in that analysis.

To better understand the origins and identities of invasive *Salsola* spp. in North America, genetic variation has been explored to a limited extent with molecular markers. In California, Ryan and Ayres (2000) used isoenzymes and RAPD (Randomly Amplified Polymorphic DNA) markers to examine genetic variation in entities that were then thought to all be *S. tragus*. Two widespread genetic entities were found within the state, distinguished initially by either molecular marker system and tepal-wing width. *Salsola tragus* Type A could be distinguished from *S. tragus* Type B by patterns of aspartate aminotransferase and 6-phosphogluconate dehydrogenase. Isoenzyme patterns were consistent with *S. tragus* Type B being diploid, and *S. tragus* Type A being tetraploid. The RAPD analyses indicated that there was more variation within *S. tragus* Type A than within *S. tragus* Type B. Also, *S. tragus* Type B was characterized by somewhat broader wings on the fruit (Ryan and Ayres 2000). The authors suggested that *S. tragus* Type B was probably a previously unrecognized taxon, due to the large genetic distance between it and *S. tragus* Type A, and their morphological and cytological differences. *Salsola tragus* Type B was thus designated as just Type B from then on.

Accessions of *S. tragus* or *S. kali* from Turkey and France fell within the general *S. tragus* Type A group according to the RAPD analysis (Ryan and Ayres 2000). Also, individuals of *S. paulsenii* were more closely associated with *S. tragus* Type A than with Type B. Later work demonstrated that *S. tragus* from Ukraine showed identical patterns of aspartate aminotransferase and 6-phosphogluconate dehydrogenase as *S. tragus* Type A from California (F. J. Ryan and S. L. Mosyakin, personal observations). RAPD analysis of samples of *S. tragus* from Ukraine (F. J. Ryan and S. L. Mosyakin personal observations) and from Uzbekistan (Sobhian et al. 2003) indicated that these were similar to *S. tragus* Type A from California as well and distinct from Type B.

The inability to determine the origin of Type B is problematic for biological control efforts. *Salsola tragus* and Type B have displayed differential susceptibility to the potential biological control fungus *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz. and the gall midge *Desertovellum stackelbergi* Mamaev (Bruckart et al. 2003; Sobhian et al. 2003; respectively), with Type B being much less affected by either agent. If a biological control agent is effective against one invasive type but not the other, there is potential for the uncontrolled type to increase its range, as happened with ecotypes of *Chondrilla juncea* L. (Burdon et al. 1981). Determining the origin of Type B may assist in locating control agents that are more effective against that entity.

Control of Type B is important, as it is the dominant *Salsola* found in the South Coast Ranges and the foothills of the Transverse and Peninsular Ranges of California. Type B is also common in the southern San Joaquin Valley of California, and while not dominant, it is almost as frequently encountered there as is *S. tragus* (P. Akers personal communication). The distribution of Type B in western North America outside of California and Arizona is unknown, as is the introduction date.

The most recent advance in understanding the identity of Type B came in 2005, when G.F. Hrusa examined a Namibian specimen of *S. kali* subsp. *austroafricana* Aellen (*Seydel 3218*, UC M308692) and noted that it closely matched Type B in all morphological characters (G.F. Hrusa, in prep.).

Molecular markers are a valuable tool for testing hypotheses of relationship and identity based on morphology. Here we use an intron of the nuclear DNA sequence for the enzyme phosphoenolpyruvate carboxylase (PEPC, E.C.4.1.1.31) to: (i) investigate the genetic identity of *S. tragus* in its native range and in North America, (ii) explore the relationships between *S. tragus*, *S. kali* subsp. *austroafricana*, and Type B, and (iii) investigate origins of Type B. This genetic analysis will aid in the development of a biological control strategy for these important weeds.

Methods

Plant Material

A total of 89 plants were sequenced for the PEPC fourth intron, including *S. soda* (USA, 1

FIG. 1. Map of western USA collections of *Salsola* spp. For many locations more than one plant was collected (see Table 1).

plant), *S. paulsenii* (USA, 2), *S. tragus* (USA, 15; Eurasia, 26; northern Africa, 4), Type B (USA, 33), and *S. kali* subsp. *austroafricana* (southern Africa, 8). More in-depth sampling was used for *S. tragus* and Type B to investigate variation within the invasion (Fig. 1). The number of Old World *S. kali* subsp. *austroafricana* samples included was limited due to its recent inclusion in the study and lack of sample availability. Small branches of dried plants were shipped to Fresno from overseas. Locations of collections, collectors, and voucher information are provided in Table 1. For a few samples, only enough material for DNA extraction was collected, and these have no herbarium voucher. All existing vouchers were deposited at CDA (California Department of Food and Agriculture, Sacramento, CA).

DNA Analysis

Following extraction of DNA by standard methods (as described in Ryan and Ayers 2000), amplification of the intron between the fourth and fifth exon of the PEPC gene utilized the primer pair ppcx4f (5′-ACTCCACAGGATGAGATGAG-3′) and ppcx5r (5′-GCAGCCATCATTCTAGCCAA-3′) designed by J.F. Gaskin from the sequences of other taxa of the Caryophyllales found in GenBank. Amplification was conducted after a 2 min denaturation at 95°C and consisted of 30 cycles of 95°C (1 min), 52°C (1 min) and 72°C (2 min); followed by 5 min at 32°C. The two PCR products (one band approximately 500 bases in length and the other approx. 400 bases in length) were present in all samples. These bands were separated by electrophoresis on a 2% agarose gel and the shorter band was excised (the identity of the longer band is unknown, and its sequence variation was not useful for this analysis). DNA was purified with the Qiagen QIAquick Gel Extraction Kit. The resultant template was sequenced on a Beckman CEQ 2000XL using reagents and protocols supplied by the manufacturer and the same primers mentioned above. Each heterozygotic genotype was cloned and sequenced to determine the haplotypes involved. Clones were created using the Promega pGEM-T Vector System II, then sequenced using the protocol above. Sequences were aligned by hand using SE-Al software (Rambaut 1996) and are available in GenBank (accession numbers are in Table 2). Haplotypes were arranged manually into a most parsimonious network (Fig. 2). Measures of haplotype diversity were based on the formula $h = (1 - \sum x_i^2)\, n/(n - 1)$, where x_i is the frequency of a haplotype and n is the sample size (Nei and Tajima 1981).

RESULTS AND DISCUSSION

DNA Sequences of *Salsola* spp.

The PEPC intron marker for taxa in this study is 394 to 396 bases in length, with 38 (9.6%) variable bases. Plant samples contained one or two discernable copies of the marker (homozygous or heterozygous, respectively). The most parsimonious network (Fig. 2), excluding the outgroup *S. soda*, contained 13 mutations with no homoplasy (i.e., no mutations had to be placed in more than one position in the network). All mutations were single nucleotide changes (no insertion-deletion events). *Salsola soda* was 25 mutational steps (23 single nucleotide changes and two insertion-deletion events) away from the cluster formed by the relatively closely related *S. tragus*, *S. kali* subsp. *austroafricana*, *S. paulsenii* and Type B.

For the 88 samples of *S. tragus, S. kali* subsp. *austroafricana, S. paulsenii* and Type B that were sequenced, we found 11 haplotypes in 15 different genotypic combinations (Table 1). Only two samples of *S. paulsenii* were examined and they each contained genotypes 1/7. This combination was not found in any other taxon sampled. The most common genotypes within *S. tragus* were 2/5 (40%) and 1/2 (28%). Both genotypes were found in Eurasia, northern Africa, and the USA.

TABLE 1. MORPHOLOGICAL AND GENOTYPIC DESCRIPTION OF SPECIMENS USED IN STUDY OF *SALSOLA*. * indicates that herbarium voucher does not exist.

Species or type	Continent	Country or U.S. state	City or location	Plant DNA #	PEPC genotype	Collector	Date of collection
S. tragus	Africa	Tunisia	Kasserine	S4-14	1/6	R. Sobhian	1-Sep-1998
S. tragus	Africa	Tunisia	Bengaden	S4-13	2/5	R. Sobhian	31-Aug-1998
S. tragus	Africa	Tunisia	Sousse	S9-43	2/11	R. Sobhian	22-Aug-1998
S. tragus	Africa	Tunisia	Sfax	S9-44	9/11	R. Sobhian	30-Aug-1998
S. tragus	Asia	China	Beijing	S4-04	9/9	R. Sobhian	25-Jul-1997
S. tragus	Asia	China	Tang Shan	S4-02	10/10	R. Sobhian	30-Jul-1997
S. tragus	Asia	China	Tang Shan	S4-03	10/10	R. Sobhian	30-Jul-1997
S. tragus	Asia	Kazakhstan	Taraz	S9-9	1/1	R. Sobhian	10-Aug-1998
S. tragus	Asia	Kazakhstan		S4-11*	1/2	R. Sobhian	1-Aug-1998
S. tragus	Asia	Kazakhstan		S6-40	1/2	L. Fornaseri	
S. tragus	Asia	Kazakhstan	S. of Cimkent	S4-10	2/5	R. Sobhian	5-Dec-1998
S. tragus	Asia	Pakistan	Warsal	S6-42	1/2	R. Sobhian	5-Aug-1998
S. tragus	Asia	Pakistan	Chashma	S9-41	1/12	R. Sobhian	5-Dec-1998
S. tragus	Asia	Uzbekistan	Sherobod	S4-5	2/5	R. Sobhian	22-Jun-1997
S. tragus	Asia	Uzbekistan	Bukhara	S4-7	2/5	R. Sobhian	24-Jun-1997
S. tragus	Europe	France	Grau du Roi	S9-15	1/2	R. Sobhian	6-Sep-1995
S. tragus	Europe	France	Carnon	S4-17	2/5	R. Sobhian	13-Oct-1995
S. tragus	Europe	Turkey	Aydincik	S6-38	1/2	R. Sobhian	27-May-1997
S. tragus	Europe	Turkey	Isparta	S8-24	1/2	R. Sobhian	14-Sep-1995
S. tragus	Europe	Turkey	Finike	S4-1	2/5	R. Sobhian	11-Sep-1997
S. tragus	Europe	Turkey	Isparta	S8-23	2/5	R. Sobhian	1-Oct-1995
S. tragus	Europe	Turkey	Aydincik	S9-37	2/5	R. Sobhian	27-May-1997
S. tragus	Europe	Turkey	Kirka	S8-22	2/6	R. Sobhian	13-Sep-1995
S. tragus	Europe	Ukraine	Genichesk Dist.	2003	2/2	I. I. Moisienko	17-Oct-2000
S. tragus	Europe	Ukraine	Gola Prystan'	2007	2/2	O. Y. Umanets	15-Oct-2000
S. tragus	Europe	Ukraine	Kiev	2000	2/5	S. Mosyakin	14-Sep-2000
S. tragus	Europe	Ukraine	Kiev	2001*	2/5	O. S. Sakun	14-Sep-2000
S. tragus	Europe	Ukraine	Gladkovka	2002	2/5	O. Y. Umanets	17-Oct-2000
S. tragus	Europe	Ukraine	Tsyurupinsk	2004	2/5	O. Y. Umanets	17-Oct-2000
S. tragus	Europe	Ukraine	Tsyurupinsk	2005	2/5	O. Y. Umanets	17-Oct-2000
S. tragus	N. America	CA	Davis	372	1/2	F. Ryan	11-Oct-1999
S. tragus	N. America	CA	Fresno	373*	1/2	F. Ryan	20-Apr-1997
S. tragus	N. America	CA	Coalinga	375	1/2	F. Ryan	23-Oct-1999
S. tragus	N. America	CA	Sacramento	4135	1/2	P. Akers RT05-3	18-Sep-2002
S. tragus	N. America	CA	Turlock	4137	1/2	P. Akers RT42-3	11-Nov-2002
S. tragus	N. America	CA	Kamm Rd./I-5	S8-19*	1/2	F. Ryan	
S. tragus	N. America	CA	Kamm Rd./I-5	S8-20*	1/2	F. Ryan	
S. tragus	N. America	CA	Fresno	374	2/2	F. Ryan	1-Oct-1999
S. tragus	N. America	CA	Davis	4132	2/5		1-Sep-2000
S. tragus	N. America	CA	Parlier	4133	2/5	P. Akers RT 14-01	23-Sep-2002
S. tragus	N. America	CA	Turlock	4136	2/5	P. Akers RT 42-01	11-Nov-2002
S. tragus	N. America	CA	Kamm Rd./I-5	S4-18*	2/5	F. Ryan	
S. tragus	N. America	WA	Spokane	S6-45	1/2	D. Ayres	11-Aug-1998
S. tragus	N. America	WA	Spokane	S9-46	2/5	D. Ayres	11-Aug-1998
S. tragus	N. America	WA	Spokane	S9-48	2/11	D. Ayres	11-Aug-1998
Type B	N. America	AZ	Avondale	6061	3/3	D. Ayres	28-Dec-1998
Type B	N. America	AZ	Phoenix	6064	3/3	D. Ayres	27-Dec-1998
Type B	N. America	AZ	Phoenix	6065	3/3	D. Ayres	27-Dec-1998
Type B	N. America	AZ	Phoenix	6067	3/3	D. Ayres	27-Dec-1998
Type B	N. America	AZ	Phoenix	S8-25	3/3	D. Ayres	27-Dec-1998

TABLE 1. CONTINUED.

Species or type	Continent	Country or U.S. state	City or location	Plant DNA #	PEPC genotype	Collector	Date of collection
Type B	N. America	AZ	Phoenix	S8-26	3/3	D. Ayres	27-Dec-1998
Type B	N. America	AZ	Tempe	S9-49	3/3	D. Ayres	27-Dec-1998
Type B	N. America	AZ	Phoenix	6066	3/4	D. Ayres	27-Dec-1998
Type B	N. America	AZ	Avondale	6060	4/4	D. Ayres	28-Dec-1998
Type B	N. America	AZ	Avondale	6062	4/4	D. Ayres	28-Dec-1998
Type B	N. America	AZ	Avondale	6063	4/4	D. Ayres	28-Dec-1998
Type B	N. America	AZ	Quartzite	S9-31	4/4	D. Ayres	28-Dec-1998
Type B	N. America	AZ	Avondale	S9-35	4/4	D. Ayres	28-Dec-1998
Type B	N. America	AZ	Quartzite	S9-36	4/4	D. Ayres	28-Dec-1998
Type B	N. America	CA	Fresno	376	3/3	F. Ryan	21-Jul-1997
Type B	N. America	CA	Famoso	4142	3/3	P. Akers RT74-01	1-Oct-2002
Type B	N. America	CA	San Diego	S9-54	3/3	F. Ryan	5-Nov-1999
Type B	N. America	CA	Maricopa	6140.00	3/3	G.F. Hrusa 16172	12-Oct-2003
Type B	N. America	CA	Paicines	6144.00	3/3	G.F. Hrusa 16186	14-Oct-2003
Type B	N. America	CA	Lockwood	6145.00	3/3	G.F. Hrusa 16193	14-Oct-2003
Type B	N. America	CA	San Lucas	6151.00	3/3	G.F. Hrusa 16183a	13-Oct-2003
Type B	N. America	CA	San Lucas	6154.00	3/3	G.F. Hrusa 16183d	13-Oct-2003
Type B	N. America	CA	Fresno	377	4/4	F. Ryan	20-Apr-1997
Type B	N. America	CA	Santa Nella	378*	4/4	F. Ryan	
Type B	N. America	CA	CA 152, Merced Co.	4140	4/4	P. Akers RT13-01	23-Sep-2002
Type B	N. America	CA	CA 152/ I-5	4141	4/4	P. Akers RT30-02	10-Oct-2002
Type B	N. America	CA	Santa Nella	S9-52*	4/4	D. Ayres	
Type B	N. America	CA	Santa Nella	S9-53*	4/4	D. Ayres	
Type B	N. America	CA	Calabasas	6168.00	4/4	B. Villegas 247-1	21-Aug-2002
Type B	N. America	CA	Calabasas	6169.00	4/4	B. Villegas 247-2	21-Aug-2002
Type B	N. America	CA	Calabasas	6170.00	4/4	B. Villegas 247-3	21-Aug-2002
Type B	N. America	CA	Calabasas	6171.00	4/4	B. Villegas 247-4	21-Aug-2002
Type B	N. America	CA	Calabasas	6172.00	4/4	B. Villegas 247-5	21-Aug-2002
S. kali subsp. *austroafricana*	Africa	South Africa	Robertson	6068	3/3	S. Neser	
S. kali subsp. *austroafricana*	Africa	South Africa	Robertson	6069	3/3	S. Neser	
S. kali subsp. *austroafricana*	Africa	South Africa	Vioolsdrift	6070	3/3	S. Neser	
S. kali subsp. *austroafricana*	Africa	South Africa	Vioolsdrift	6071	3/3	S. Neser	
S. kali subsp. *austroafricana*	Africa	Namibia	Windhoek	6797	3/3	A. Kirk & C. Pickett	5-Sep-2005
S. kali subsp. *austroafricana*	Africa	South Africa	Kimberly #3	6800	3/3	M. Rejmanek	
S. kali subsp. *austroafricana*	Africa	South Africa	Kimberly	6802	3/3	M. Rejmanek	28-Oct-2005
S. kali subsp. *austroafricana*	Africa	South Africa	Kimberly	6803	3/3	M. Rejmanek	28-Oct-2005
S. paulsenii	N. America	CA	Eureka Valley	Eureka 3	1/7	F. Ryan	28-Dec-2001
S. paulsenii	N. America	CA	Eureka Valley	Eureka 4	1/7	F. Ryan	28-Dec-2001
S. soda	N. America	CA	Berkeley	4160	15/15	F. Ryan	13-Aug-1998

TABLE 2. GENBANK ACCESSION NUMBERS FOR *SALSOLA* SPP. HAPLOTYPES.

PEPC haplotype	GenBank accession number
1	DQ257378
2	DQ005542
3	DQ257379
4	DQ005543
5	DQ005544
6	DQ257380
7	DQ257381
9	DQ257382
10	DQ257383
11	DQ257384
12	DQ257385
15	DQ257386

The most common *S. tragus* genotype among the USA specimens was 1/2 (53%), which was found both in the central valley of California and in Spokane, WA. The 1/2 genotype was found in Turkey, Kazakhstan, Pakistan, and presumably in intermediate localities while 2/5 was found in Tunisia, Turkey, Kazakhstan, Uzbekistan, and Ukraine. Due to the widespread distribution in the native range of the genotypes found in the USA, conclusions cannot be drawn concerning the native source of introduced *S. tragus*.

The haplotypes found in *S. tragus*, *S. kali* subsp. *austroafricana*, Type B, and *S. paulsenii* are all relatively closely clustered on the haplotype network (Fig. 2) when compared to the

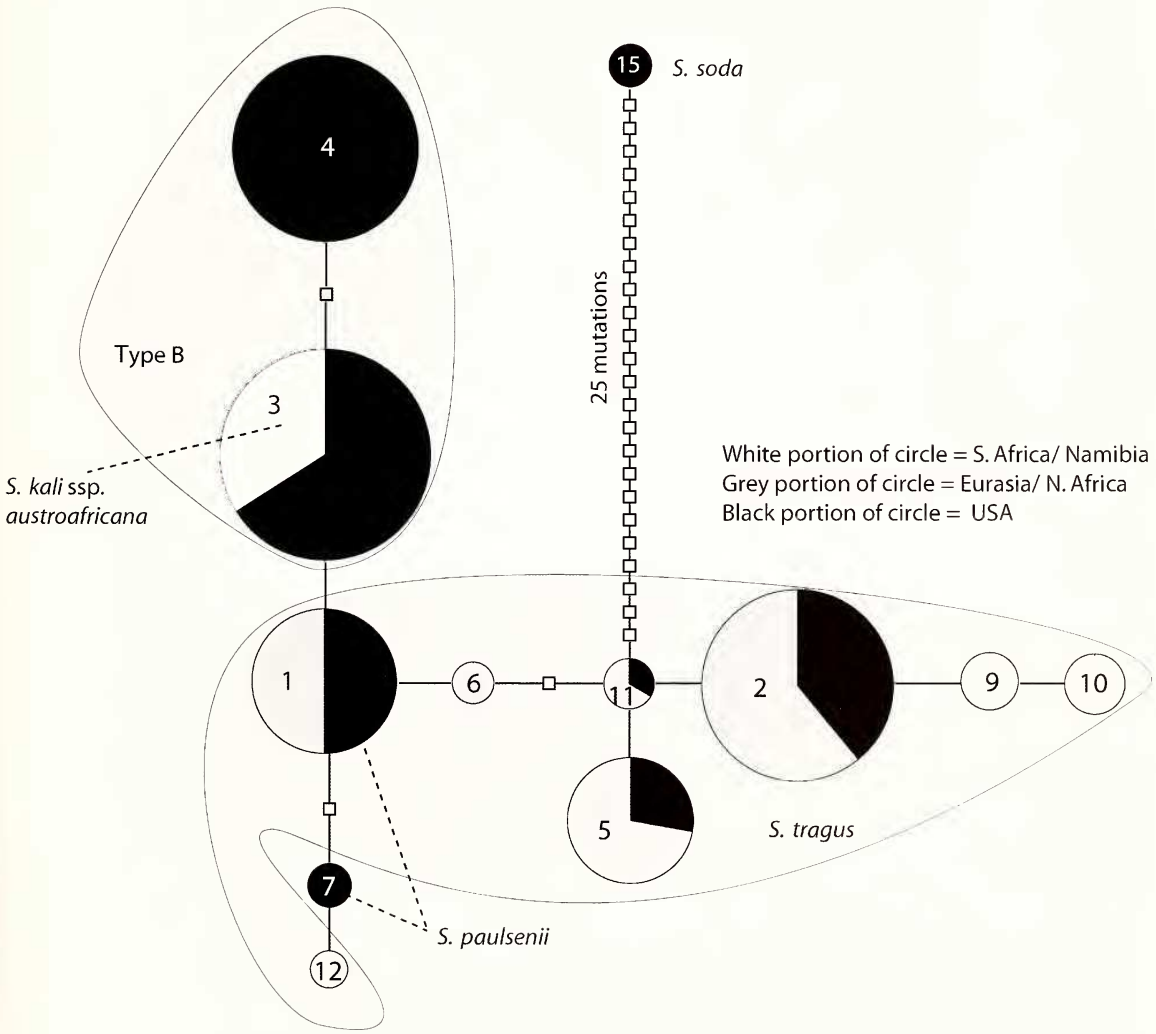

FIG. 2. Haplotype network of DNA sequences of the fourth intron of the PEPC gene region for 89 samples of *Salsola* species from the USA, Africa and Eurasia. Circles represent haplotypes recovered, and squares along lineages in between circles indicate haplotypes not recovered. Size of a circle is proportionate to haplotype frequency worldwide. The pie slices of a circle indicate the percentage of haplotypes from the USA (black), Eurasia/ N. Africa (gray), and southern Africa (white). Each link between haplotypes indicates one mutational event. Angle of bifurcation and length of link between haplotypes have no significance. The loops that surround portions of the haplotype network indicate taxonomic status of plant collections sampled.

mutational distance to the congener *S. soda*. *Salsola tragus* and *S. paulsenii* share haplotype 1, which may be due to incomplete lineage sorting or hybridization, either of which suggests a close relationship of the two species. The native ranges of *S. tragus* and *S. paulsenii* overlap and morphological hybrids appear to be present (Rilke 1999). Further analysis could investigate if putative hybrid combinations in the USA and native range are genetically similar, or if novel hybrid genotypes have been created in the USA since the introduction of the two species, which has happened in other taxa (e.g., Ellstrand and Schierenbeck 2000; Gaskin and Schaal 2002).

Haplotype diversity was larger in the *S. tragus* invasion compared to the Type B invasion in the USA (h = 0.739 vs. 0.506 respectively). Levels of observed heterozygosity varied greatly between invasive samples from the two taxa, with H_o = 0.84 in *S. tragus* compared to H_o = 0.03 in Type B. These results are consonant with *S. tragus* being tetraploid (2N = 36), and Type B being a diploid species (2N = 18) which is supported by the isoenzyme patterns as well (Ryan and Ayres 2000), but could also be influenced by the founding event(s). Each of the Type B genotypes appeared to be quite widespread, in agreement with initial observations of a low amount of genetic variation in comparison with *S. tragus* (Ryan and Ayres 2000).

The interaction of biological control agents with different *S. tragus* genotypes has not been examined. Preliminary host-specificity results with *Colletotrichum gloeosporioides* (Bruckart et al. 2003) and *Desertovellum stackelbergi* (Sobhian et al. 2003) indicate that plant genotype may be a factor in certain *Salsola* control scenarios. Though distinct genotypes of invasives may not correlate with the phenotypic characters that influence behavior of biological control agents (see Reed and Frankham 2001), there are examples of intraspecific genetic variation of weeds affecting insect herbivory in other systems (Karban and Kittelson 1999; Herrin and Warnock 2002). Inclusion of a variety of invasive *S. tragus* genotypes in biological control agent host-specificity tests, compared to using material from just one or a few stock individuals, will extend our understanding of how agents might be effective across the range of the invasion.

Analysis of *S. tragus* and Type B with the PEPC intron marker confirms the earlier morphological, cytological, isoenzymic, and RAPD results supporting *S. tragus* and Type B as distinct entities. The recent inclusion of *S. kali* subsp. *austroafricana* from southern Africa in our studies provides the first match with Type B using either morphology or DNA. Although it is possible that other *Salsola* taxa in the Old World may also possess the same haplotypes as Type B, the concurrent morphological and molecular matches indicate that California and Arizona's Type B is indeed the plant described as *S. kali* subsp. *austroafricana*. Haplotype 4 of Type B still does not match with any Old World specimens, and further sampling needs to be done on *S. kali* subsp. *austroafricana* in southern Africa to determine if genotype 3/3 is dominant in the subspecies. *Salsola kali* subsp. *austroafricana* has also been reported from Australia (Rilke 1999), but no specimens were cited and it has not been recently confirmed from there.

Salsola sect. *Kali* (which includes *S. kali* subsp. *austroafricana*) has usually been assumed introduced in southern Africa, as the section is thought to be native only in the northern hemisphere. Botschantsev (1974) treated *S. kali* ssp. *austroafricana* as a synonym of *S. australis* R.Br. (=*S. tragus* L.) and stated that these were adventive in south and southwest Africa. Rilke (1999) also considered section *Kali* introduced in the southern Africa region. However, given the specific epithet, this position must not have been universal among all botanists. Perusal of large European and Asian herbaria (LE, KW, and MPU) by Ukrainian and USDA collaborators, and the accounting by Rilke, have not revealed specimens from outside of southern Africa that are morphologically similar to *S. kali* ssp. *austroafricana*. Known native *Salsola* in southern Africa are not closely related to the *S. tragus* complex (Botschantsev 1974) and a single intraspecific level hemispheric disjunct, particularly of a weedy plant, would seem unusual. However, given its apparent absence in European and Asian collections, it cannot be stated with absolute confidence that the plant is introduced in southern Africa. Further study would appear necessary before this question can be answered.

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